

Supplementary Materials

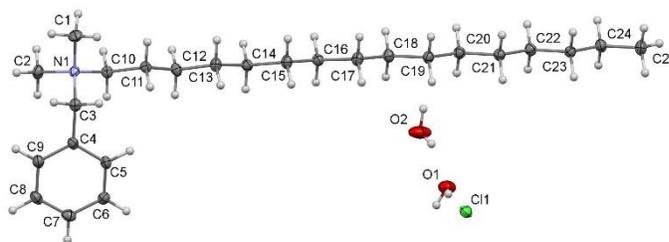


Figure S1. Three-dimensional structure of BAC₁₆, as determined by single-crystal X-ray diffraction (image courtesy of Dr. Muhammed Yousufuddin and with permission from IUCr Journals). Compound purification and identification methods and data were previously published by Aguinaga et al. (2023) [30]. The crystal contains BAC₁₆ and two molecules of water.

Table S1. P10-Control Strain MICs for BAC₁₂₋₁₄ and BAC₁₆.

MIC (mg/L)	BAC ₁₂₋₁₄ (P10-Control)	P value (P0 vs. P10-Control)	BAC ₁₆ (P10-Control)	P value (P0 vs. P10-Control)
<i>S. epidermidis</i>	0.28 ± 0.04	0.0901	0.25 ± 0.05	0.4226
<i>C. xerosis</i>	0.56 ± 0.11	0.8407	0.40 ± 0.13	0.7217
<i>S. aureus</i>	0.69 ± 0.24	0.5899	0.30 ± 0.12	0.7384
<i>K. pneumoniae</i>	2.56 ± 0.17	0.4833	0.63 ± 0.22	0.5057
<i>E. coli</i>	4.38 ± 0.48	0.6420	1.88 ± 0.22	0.5536
<i>P. aeruginosa</i>	13.13 ± 2.42	0.6186	21.25 ± 2.17	0.3343

MIC values are shown as mean ± standard deviation. Mean values include a minimum of two independent experiments with three replicates per experiment, for eight bacterial isolates per species. P10-Control, progenitor strains were passaged alongside experimental groups but were not exposed to sublethal BAC concentrations. Two-tailed t-tests were used to compare P0 (see Table 1) and P10-Control MIC values for each bacterial species. P values are shown. All p values were greater than 0.05 and were therefore not statistically significant ($p < 0.05$).

Table S2. Individual comparisons of BAC₁₂₋₁₄ and BAC₁₆ P0 and P10 MICs.

P values	P0 MIC	P10 MIC
	BAC ₁₂₋₁₄ vs BAC ₁₆	BAC ₁₂₋₁₄ vs BAC ₁₆
<i>S. epidermidis</i>	0.6616	0.1971
<i>C. xerosis</i>	0.0049	1.50 × 10⁻⁸
<i>S. aureus</i>	0.0004	6.70 × 10⁻¹⁴
<i>K. pneumoniae</i>	4.40 × 10⁻⁹	2.91 × 10⁻¹⁶
<i>E. coli</i>	3.78 × 10⁻⁹	6.80 × 10⁻¹⁰
<i>P. aeruginosa</i>	0.0002	1.57 × 10⁻¹¹

Two-tailed t-tests were used to compare P0 and P10 MIC values for each BAC compound. P values are shown. P values in bold are statistically significant ($p < 0.05$).

Table S3. Minimum Bactericidal Concentrations (MBCs) for BAC₁₂₋₁₄ and BAC₁₆.

MBC (mg/L)	BAC ₁₂₋₁₄	BAC ₁₆	P value (BAC ₁₂₋₁₄ vs. BAC ₁₆)
<i>S. epidermidis</i>	0.47 ± 0.08	0.63 ± 0.22	0.0960

<i>C. xerosis</i>	1.03 ± 0.26	0.88 ± 0.22	0.2453
<i>S. aureus</i>	1.63 ± 0.22	0.47 ± 0.08	2.73 × 10⁻⁹
<i>K. pneumoniae</i>	3.44 ± 0.39	1.06 ± 0.11	3.26 × 10⁻¹⁰
<i>E. coli</i>	9.25 ± 0.97	2.75 ± 0.97	5.20 × 10⁻⁹
<i>P. aeruginosa</i>	18.75 ± 3.31	45.63 ± 7.68	6.68 × 10⁻⁷

MBC values are shown as mean ± standard deviation. Mean values include a minimum of two independent experiments with three replicates per experiment, for eight bacterial isolates per species. Two-tailed t-tests were used to compare BAC₁₂₋₁₄ and BAC₁₆ MBC values for each bacterial species. P values are shown. Values in bold are statistically significant ($p < 0.05$).

Table S4. Comparison of MBCs across all bacterial species.

P values	BAC ₁₂₋₁₄			BAC ₁₆		
	One-way ANOVA	Levene's test	Welch F test	One-way ANOVA	Levene's test	Welch F test
MBCs	5.31 × 10⁻²⁷	2.37 × 10⁻⁸	2.526 × 10⁻¹⁵	2.10 × 10⁻²⁹	7.06 × 10⁻¹²	1.70 × 10⁻¹¹

One-way ANOVA analyses were performed to test for differences in BAC₁₂₋₁₄ and BAC₁₆ MBCs across all bacterial species. Levene's test was used to determine homogeneity of variance from the means. For all comparisons Levene's test p values were significant ($p < 0.05$), therefore the Welch F test was used to address unequal variance. P values for each analysis are shown. Values in bold are considered statistically significant ($p < 0.05$).

Table S5. Comparison of P0 MICs and P10 MICs across all bacterial species.

P values	BAC ₁₂₋₁₄			BAC ₁₆		
	One-way ANOVA	Levene's test	Welch F test	One-way ANOVA	Levene's test	Welch F test
P0 MIC Values	7.03 × 10⁻²⁹	5.71 × 10⁻⁹	3.40 × 10⁻¹⁴	1.24 × 10⁻³⁴	0.0900	1.43 × 10⁻¹⁴
P10 MIC Values	2.98 × 10⁻⁴⁴	4.92 × 10⁻¹⁹	3.28 × 10⁻²⁰	1.38 × 10⁻²⁸	6.59 × 10⁻⁵	4.51 × 10⁻¹⁴

One-way ANOVA analyses were performed to test for differences in P0 MICs and P10 MICs across all bacterial species for each BAC. Levene's test was used to determine homogeneity of variance from the means. For all comparisons Levene's test p values were significant ($p < 0.05$), therefore the Welch F test was used to address unequal variance. P values for each analysis are shown. Values in bold are considered statistically significant ($p < 0.05$).

Table S6. Individual comparisons of MICs between bacterial species.

BAC ₁₂₋₁₄ P0 MIC	<i>S. epidermidis</i>	<i>C. xerosis</i>	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>E. coli</i>
<i>C. xerosis</i>	4.71 × 10⁻⁶	---	---	---	---
<i>S. aureus</i>	5.48 × 10⁻⁵	0.5457	---	---	---
<i>K. pneumoniae</i>	4.73 × 10⁻¹⁰	5.19 × 10⁻⁹	1.20 × 10⁻⁸	---	---
<i>E. coli</i>	2.16 × 10⁻¹²	9.42 × 10⁻¹²	1.70 × 10⁻¹¹	4.02 × 10⁻⁶	---
<i>P. aeruginosa</i>	1.42 × 10⁻¹⁰	2.03 × 10⁻¹⁰	2.19 × 10⁻¹⁰	2.57 × 10⁻⁹	2.79 × 10⁻⁸
BAC ₁₂₋₁₄ P10 MIC	<i>S. epidermidis</i>	<i>C. xerosis</i>	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>E. coli</i>
<i>C. xerosis</i>	2.91 × 10⁻⁹	---	---	---	---
<i>S. aureus</i>	4.51 × 10⁻¹⁴	8.89 × 10⁻¹²	---	---	---
<i>K. pneumoniae</i>	2.01 × 10⁻¹⁶	5.45 × 10⁻¹⁶	1.48 × 10⁻¹⁴	---	---
<i>E. coli</i>	2.92 × 10⁻¹⁰	4.37 × 10⁻¹⁰	1.54 × 10⁻⁹	2.63 × 10⁻⁶	---
<i>P. aeruginosa</i>	7.14 × 10⁻¹⁶	7.70 × 10⁻¹⁶	9.62 × 10⁻¹⁶	2.81 × 10⁻¹⁵	4.08 × 10⁻¹⁴
BAC ₁₆ P0 MIC	<i>S. epidermidis</i>	<i>C. xerosis</i>	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>E. coli</i>
<i>C. xerosis</i>	0.0535	---	---	---	---
<i>S. aureus</i>	0.2589	0.2631	---	---	---
<i>K. pneumoniae</i>	2.13 × 10⁻⁶	0.0054	8.36 × 10⁻⁵	---	---

<i>E. coli</i>	1.81 × 10⁻¹³	1.20 × 10⁻¹¹	1.06 × 10⁻¹²	3.30 × 10⁻¹¹	---
<i>P. aeruginosa</i>	5.89 × 10⁻¹²	6.45 × 10⁻¹²	6.08 × 10⁻¹²	7.44 × 10⁻¹²	2.04 × 10⁻¹¹
BAC ₁₆ P10 MIC	<i>S. epidermidis</i>	<i>C. xerosis</i>	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>E. coli</i>
<i>C. xerosis</i>	5.11 × 10⁻⁶	---	---	---	---
<i>S. aureus</i>	0.0939	0.0342	---	---	---
<i>K. pneumoniae</i>	3.34 × 10⁻⁷	0.0006	6.54 × 10⁻⁵	---	---
<i>E. coli</i>	4.95 × 10⁻¹⁰	2.19 × 10⁻⁹	1.44 × 10⁻⁹	2.05 × 10⁻⁸	---
<i>P. aeruginosa</i>	5.81 × 10⁻¹⁴	6.00 × 10⁻¹⁴	5.90 × 10⁻¹⁴	6.26 × 10⁻¹⁴	8.38 × 10⁻¹⁴

Two-tailed t-tests were used to identify differences in MICs between individual bacterial species. P values are shown.

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Values in bold are statistically significant ($p > 0.05$).

Table S7. Comparison of P10+EPI MICs to P0 MICs and P10 MICs.

P values	BAC ₁₂₋₁₄		BAC ₁₆	
	vs. P0	vs. P10	vs. P0	vs. P10
<i>S. epidermidis</i>	NT	NT	NT	NT
<i>C. xerosis</i>	0.0867	4.71 × 10⁻⁶	NT	NT
<i>S. aureus</i>	0.7900	2.60 × 10⁻¹²	NT	NT
<i>K. pneumoniae</i>	6.81 × 10⁻⁷	2.04 × 10⁻⁸	NT	NT
<i>E. coli</i>	1.12 × 10⁻¹¹	0.0001	NT	NT
<i>P. aeruginosa</i>	3.95 × 10⁻⁹	2.62 × 10⁻¹³	2.91 × 10⁻⁹	1.4 × 10⁻⁹

Two-tailed t-tests were used to compare MICs following EPI treatment to P0 and P10 MICs. P values are shown. Values in bold are statistically significant ($p < 0.05$). NT, Not Tested– no change in P10 MIC values were observed for BAC-acclimated bacteria (See Table 2).